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Mobilities of Organic Compounds in Reconstituted Cuticular Wax of Barley Leaves: Effects of Monodisperse Alcohol Ethoxylates on Diffusion of Pentachlorophenol and Tetracosanoic Acid

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Abstract: Effects of monodisperse alcohol ethoxylates on mobilities of ^{14}C-labelled pentachlorophenol (PCP) and tetracosanoic acid ($C_{24}AC$) in reconstituted cuticular wax of barley leaves were measured. Depending on the respective alcohol ethoxylate investigated, the diffusion coefficient (D) of PCP in barley wax was increased by factors ranging from 3·3 to 19·6, whereas D of $C_{24}AC$, was increased by factors varying between 22 and 315. In order to analyse the relationship between the concentration of surfactants in the wax and their effects on D, the amounts of alcohol ethoxylates dissolved in the wax at equilibrium with external concentrations well above the critical micelle concentration (CMC) were determined. Wax/water partition coefficients (K_{ww}) of the alcohol ethoxylates were about one order of magnitude lower than cuticle/water partition coefficients (K_{cw}), which is a consequence of the semi-crystalline structure of the wax compared with amorphous cutin. Correlations between effects on D and maximum amounts of alcohol ethoxylates dissolved in the wax were obtained indicating an unspecific wax/surfactant interaction. This was solely dependent on the amount of surfactant sorbed to the wax, leading to increased mobilities of pesticides in the wax. Applying ESR-spectroscopy, which gave an insight into the molecular structure of the wax, supported this interpretation of an unspecific plasticising effect of the alcohol ethoxylates on the molecular structure of the wax. The results obtained in this study are in good accordance with the results obtained in a recent study investigating the effects of the same group of alcohol ethoxylates on mobilities of pesticides in isolated, but intact, cuticular membranes of *Citrus*. This demonstrates that the investigation of isolated and subsequently reconstituted cuticular wax is a useful model system analysing the mechanisms of the surfactant interaction with the transport-limiting barrier of plant cuticles.

Key words: cuticular wax, diffusion, foliar uptake, plant cuticle, surfactant, transport

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1 INTRODUCTION

Many plant-protecting chemicals, sprayed onto leaf surfaces, have to diffuse across a transport-limiting barrier, the plant cuticle, in order to reach their site of action.[1] Plant cuticles, forming the interface between leaf and atmosphere, are composite membranes consisting of cutin and cuticular wax.[2,3] Whereas cutin establishes an amorphous three-dimensional polymer network,[4] cuticular wax is deposited into the outer region and to the outer surface of the cutin polymer.[5,6] Due to its semicrystalline nature, it is essentially the cuticular wax that forms the transport-limiting barrier of the intact plant cuticle.[7–10] In order to obtain a more fundamental and mechanistic understanding of this waxy transport barrier, an experimental system has been developed simulating cuticular transport properties with isolated and subsequently reconstituted cuticular wax.[11–13]

Surfactants are widely used as adjuvants for agrochemicals. Important reasons for their use are increased solubility of the active ingredient in the spray solution and improved wetting, spreading and adhesion of the spray droplets on leaf surfaces.[14] Furthermore, it has been shown that surfactants have the ability to increase cuticular permeability for penetrating substances, although the mechanism of this is not clear.[15–20] A study investigating the effects of monodisperse alcohol ethoxylates on the mobility of 2,4-D in isolated cuticles suggested that surfactants have to sorb to the transport-limiting wax barrier of cuticles in order to increase their permeability.[21–22]

Therefore, a study of the transport properties of isolated cuticular wax should provide information relevant to the understanding of wax/surfactant interactions. In a first attempt, the interaction of one monodisperse alcohol ethoxylate with reconstituted barley wax was investigated, showing that it is possible to simulate surfactant/cuticle interactions investigating reconstituted cuticular wax.[12] The present work investigates surfactant/wax interactions including an increased number of monodisperse alcohol ethoxylates and some further experimental procedures, which help to obtain a better understanding of the increase of cuticular permeability by surfactants.

2 MATERIALS AND METHODS

2.1 Plant materials

Leaves of four- to six-week-old barley plants (*Hordeum vulgare* L. cv. Magie), grown in growth chambers [16 h light at 500–800 μmol m^{-2} s^{-1} PAR (photosynthetic active radiation), 25°C and 50% R.H. (relative humidity); 15°C and 90% R.H. during the dark], were sampled for the experiments. As described previously, cuticular wax was extracted by immersing the leaves for 1–3 s in chloroform at room temperature.[11] Extracts were filtered twice and the solvent volume was reduced to give a final wax concentration of about 50 mg ml^{-1}. Wax solutions prepared in this way were stored at − 18°C until they were used in the experiments.

2.2 Chemicals and analytical procedures

Monodisperse alcohol ethoxylates used in the experiments (Table 1) were obtained from Fluka (Neu-Ulm, Germany). Chemical purity of the alcohol ethoxylates was better than 97% (own determination by GC-analysis). ^{14}C-labelled pentachlorophenol (PCP; specific radioactivity: 155 GBq mol^{-1}) was obtained from Sigma (Deisenhofen, Germany) and ^{14}C-labelled tetracosanoic acid (C_{24}AC; specific radioactivity: 1728 GBq mol^{-1}) was purchased at CEA (Grenoble, France). Radiochemical purities of the ^{14}C-labelled compounds were better than 98% (own determination by radio-TLC). The spin probe used in the electron spin resonance (ESR) studies was 5-doxyl stearic acid (5-SASL) containing the nitroxyl-group at carbon atom 5. The

TABLE 1
Abbreviations, Molecular Weights (MW) and Molar Volumes (MV) of the Eight Alcohol Ethoxylates Investigated in This Study

Compound[a]	*Abbreviation*	*MW (g)*	*MV (cm^3 mol^{-1})*[b]
Diethylene glycol monobutyl ether	C_4E_2	162·23	141·19
Triethylene glycol monohexyl ether	C_6E_3	234·34	203·42
Tetraethylene glycol monooctyl ether	C_8E_4	306·45	265·65
Pentaethylene glycol monodecyl ether	$C_{10}E_5$	378·56	327·88
Hexaethylene glycol monododecyl ether	$C_{12}E_6$	450·66	390·11
Octaethylene glycol monododecyl ether	$C_{12}E_8$	538·77	458·21
Heptaethylene glycol monotetradecyl ether	$C_{14}E_7$	522·77	452·34
Octaethylene glycol monohexadecyl ether	$C_{16}E_8$	594·88	514·57

[a] Source: Fluka (Neu-Ulm, Germany).
[b] Calculated according to Abraham and McGowan.[33]

spin probe, which was obtained from Sigma (Deisenhofen, Germany), had a chemical purity of 99%.

Radioactivity was measured by liquid scintillation counting (TRI Carb 2000, Canberra Packard, Frankfurt, Germany) adding an adequate amount of scintillation cocktail (Ultima Gold XR, Canberra Packard, Frankfurt, Germany) to the samples. Analysis of non-radioactive alcohol ethoxylates sorbed to barley wax was carried out by capillary gas chromatography (GC) equipped with on-column injectors and flame ionisation detectors (HP 5890 II, Hewlett-Packard, Millville, NJ, USA) as described previously.[23] Specific correction factors were determined for each homologue.

2.3 Preparation of wax samples

Wax samples used in the experiments were prepared as described previously.[11] In short, aluminium discs (100 mm^2 surface area and 25 μm thickness) were immersed in the chloroform solutions so that, after evaporation of the solvent, the discs were covered with a homogeneous layer of wax. In order to improve the adhesion of the wax to the surface of the discs, they were heated to 100°C for 5 min. The amount of wax covering the aluminium surface was determined by weight using an electronic microbalance (Sartorius, Göttingen, Germany; accuracy: ± 1 μg). The average thickness of investigated wax layers was $2{\cdot}2(\pm 1)$ μm, using a wax density of 0·9 g cm^{-3} for the calculation of the thickness.[24]

Wax samples, prepared according to the above method, will be referred to as 'non-radioactive' wax samples and were used for investigating the sorption of alcohol ethoxylates to barley wax. In addition, 'radioactive' wax samples were prepared by adding a defined amount of a ^{14}C-labelled compound (PCP or $C_{24}AC$) to the chloroform/wax solutions prior to recrystallisation and heating of the wax. 'Radioactive' wax samples were used for investigating the effects of the different alcohol ethoxylates on the diffusion of the ^{14}C-labelled compounds in recrystallised barley wax.

2.4 Determination of alcohol ethoxylate sorption in barley wax and calculation of wax/water partition coefficients

Three 'non-radioactive' wax samples were incubated in 25-ml glass vials containing an aqueous solution of the respective alcohol ethoxylates three to 25 times above their CMC (Conc; Table 2). As only small amounts of substance were sorbed in wax after equilibration, the large external aqueous volume and the relatively high surfactant concentration guaranteed that the concentration of monomeric surfactant molecules in the external aqueous solution remained nearly constant during the course of the experiment.

Equilibrium sorption of the alcohol ethoxylates between the wax samples and the external aqueous solutions was established after 24 h. Wax samples were removed from the surfactant solutions, dipped for a few seconds into deionised water to remove superficially adhering alcohol ethoxylate and blotted carefully with a cellulose tissue to remove adhering water. Each wax sample was extracted with chloroform (1 ml; 30 min; 70°C), the aluminium disc removed and the chloroform evaporated. The residue was treated at 70°C with pyridine (5 μl) and *N,N*-bis-trimethylsilyltrifluoroacetamide (BSTFA; 5 μl; Machery-Nagel, Düren, Germany), which converts the free hydroxyl and carboxyl groups into their trimethylsilyl ethers and esters, respectively. Finally, samples were redissolved in chloroform containing a defined amount of hexacosan-1-ol (Fluka, Neu-Ulm, Germany) as internal standard. One microlitre of each sample was analysed by capillary gas chromatography (HP 5890 II, Hewlett-Packard, Millville, NJ, USA). Peaks of the respective alcohol ethoxylates, which could clearly be separated in the chromatogram from the peaks of the wax constituents, were quantified by internal standardisation using hexacosan-1-ol.

Wax/water partition coefficients of the alcohol ethoxylates were calculated according to:

$$K_{ww} = \frac{M_{wax}/m_{wax}}{M_{water}/m_{water}} \quad (1)$$

M represents the amount of the investigated substance (mol) in wax and water at equilibrium sorption, and m (kg) represents the mass of the two compartments wax and water, respectively.

2.5 Desorption of ^{14}C-labelled PCP and $C_{24}AC$ from barley wax and calculation of diffusion coefficients

Desorption experiments were carried out by incubating single 'radioactive' wax samples into 5-ml glass vials containing the aqueous desorption medium. Vials were closed with screw caps and rotated (60 rev min^{-1}) in the dark at 25°C. After defined periods of time, desorption medium in each vial was replaced by fresh solution and radioactivity was determined by liquid scintillation counting of the sampled desorption solution. In a first set of experiments, desorption kinetics (relative amounts desorbed versus time) of the two ^{14}C-labelled compounds PCP and $C_{24}AC$ were determined using borate buffer (pH 9·0; 10^{-2} M), which is an inert desorption medium with no effect on wax properties.[12]

Desorption kinetics could be linearised up to 50% desorption, plotting the relative amounts desorbed from the wax samples versus the square root of time. Diffusion coefficients of the two ^{14}C-labelled compounds could be calculated from the slopes of regression lines fitted to linearised desorption kinetics applying eqn

(2):[25]

$$\frac{M_t}{M_0} = \frac{4}{\Delta x} \cdot \sqrt{\frac{D}{\pi}} \cdot \sqrt{t} \quad (2)$$

M_t/M_0 is the relative amount desorbed, Δx (m) is the thickness of the wax layer, D ($m^2\ s^{-1}$) is the diffusion coefficient and t (s) is the time. Rearranging the term $(4/\Delta x)\sqrt{D/\pi}$, which is proportional to the slope of the regression line, finally allows the calculation of the molecular diffusion coefficient (D) of the respective ^{14}C-labelled compound in the wax sample.

The effects of different alcohol ethoxylates on desorption kinetics of PCP and $C_{24}AC$ were measured using aqueous solutions of the surfactants as desorption media instead of borate buffer. Concentrations of the alcohol ethoxylates were always well above their CMC (three- to 25-fold), which kept the concentration of the monomers in the desorption medium constant during the course of the experiment. Calculated effects were the ratio of the diffusion coefficients, obtained using solutions of alcohol ethoxylates as external desorption media, divided by diffusion coefficients measured using borate buffer as external desorption medium.

2.6 Electron spin resonance studies

The spin probe 5-SASL was added to the wax/chloroform solution at a concentration of 1 mg g^{-1} wax. For the investigation of the effects of the different alcohol ethoxylates, the concentration of each single alcohol ethoxylate, which had been measured by GC-analysis to be the maximum possible amount of surfactant sorbed to the wax above the CMC, was added separately to the wax/5-SASL mixture in chloroform. The solvent of the wax samples prepared in this way was evaporated under a gentle stream of nitrogen. Remaining wax crystals were transferred to glass tubes (inner diameter: 1 mm), which were sealed after the addition of the wax sample, melting the opening of the glass tube. ESR spectra were recorded on a Varian E-line ESR-spectrometer (Varian, Palo Alto, USA) at every 10°C, covering a temperature range from −10°C to a maximum of 60°C. At each temperature three spectra were recorded and averaged.

2.7 Statistics

Results, measuring sorption of different alcohol ethoxylates to wax, are based on three replicates. Diffusion coefficients of each of the two compounds and each different desorption medium are based on five replicates. Results in the tables and the figures are given as means with 95% confidence intervals, unless stated otherwise.

3 RESULTS

Maximum equilibrium concentration of alcohol ethoxylates dissolved in barley wax decreased by a factor of about 6 from 48·7 mmol kg^{-1} for C_4E_2 to 8·4 mmol kg^{-1} for $C_{16}E_8$ (Table 2). Calculated wax/water partition coefficients (K_{ww}) ranged over more than five orders of magnitude from 0·083 for C_4E_2 to 12350 for $C_{16}E_8$ (Table 2). On average, K_{ww} values, which were strongly correlated to cuticle/water partition coefficients (K_{cw}), were about one order of magnitude lower than K_{cw} values of the respective alcohol ethoxylates (Fig. 1).

The effects of the respective alcohol ethoxylates on the mobilities of PCP and $C_{24}AC$ in reconstituted barley wax reached maxima in the range C_6E_3 to

TABLE 2
Critical Micelle Concentrations (CMC), Cuticle/Water Partition Coefficients (K_{cw}), Equilibrium Concentrations in Barley Wax (CONC) and Calculated Wax/Water Partition Coefficients (K_{ww}) of the Alcohol Ethoxylates Investigated in This Study

Compound	*CMC*[a] (*mmol kg*$^{-1}$)	K_{cw}[a]	*CONC*[b,c] (*mmol kg*$^{-1}$)	K_{ww}[c]
C_4E_2	589	0·912	48·7 ± 4·3	0·083 (±0·0073)
C_6E_3	60	6·76	49·9 ± 9·7	0·83 (±0·16)
C_8E_4	6·2	50·11	30·3 ± 4·9	4·9 (±0·79)
$C_{10}E_5$	0·63	372	22·5 ± 3·4	35·7 (±5·4)
$C_{12}E_6$	0·065	2754	13·1 ± 2·4	201 (±37)
$C_{12}E_8$	0·098	1259	10·2 ± 2·6	104 (±27)
$C_{14}E_7$	0·0066	20417	10·3 ± 2·9	1561 (±439)
$C_{16}E_8$	0·00068	151356	8·4 ± 1·3	12350 (±1910)

[a] Values are calculated from prediction equations published in Riederer *et al.*[23]
[b] External aqueous concentrations of the alcohol ethoxylates were between 3 and 25 times the concentrations of the respective CMCs.
[c] Values are ±95% confidence intervals.

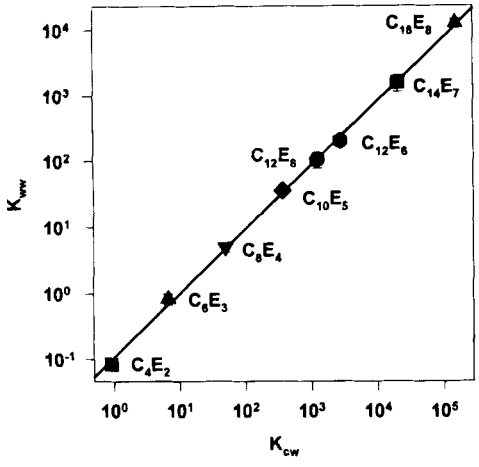

Fig. 1. Wax/water partition coefficients (K_{ww}) calculated from the maximum amount of alcohol ethoxylates sorbed in the wax and the critical micelle concentration (CMC) as a function of estimated cuticle/water partition coefficients (K_{cw}). CMC and K_{cw} values were obtained from Riederer *et al.*[23] The parameters of the linear regression equation were: log $K_{ww} = 0{\cdot}97 \times \log K_{cw} - 0{\cdot}98$ ($r^2 = 0{\cdot}999$).

$C_{10}E_5$. Effects on PCP mobility ranged from a 3·3-fold increase of D in the presence of $C_{14}E_7$ to a 19·6-fold increase of D with C_6E_3. With $C_{24}AC$, however, effects of the same alcohol ethoxylates on D were about one

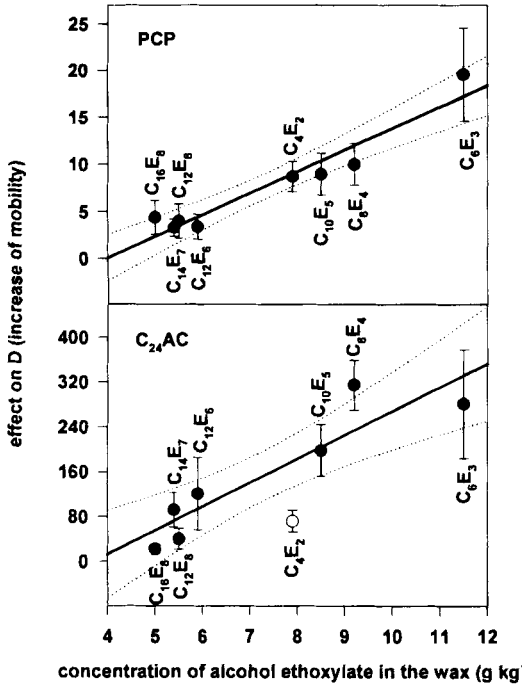

Fig. 2. Effects of alcohol ethoxylates on mobilities of pentachlorophenol (PCP) and tetracosanoic acid ($C_{24}AC$) in reconstituted wax of *Hordeum vulgare* L. as a function of the maximum equilibrium concentration of the alcohol ethoxylates in barley wax. Parameters of the linear regression equations with each of the compounds were: (1) PCP: effect = 2·29 × maximum concentration in wax − 9·08 ($r^2 = 0{\cdot}92$); (2) $C_{24}AC$: effect = 42·7 × maximum concentration in wax − 158 ($r^2 = 0{\cdot}84$).

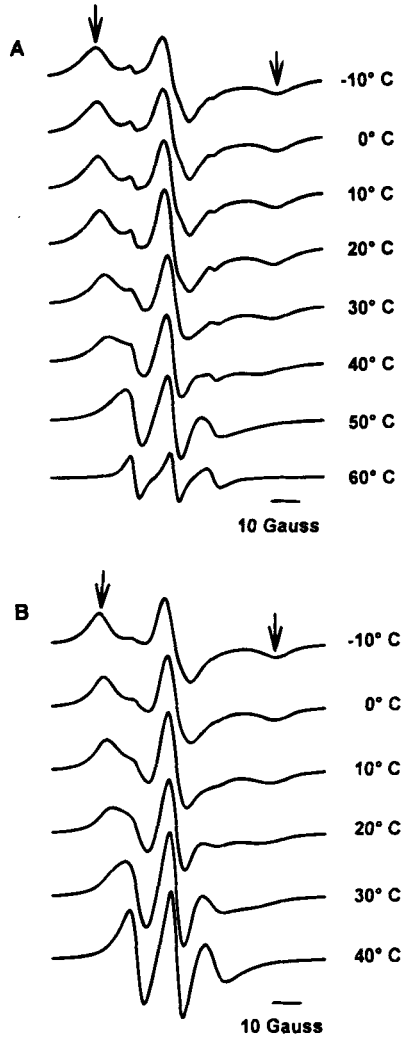

Fig. 3. Electron-spin resonance spectra of the spin label 5-doxyl stearic acid as a function of temperature in (A) pure wax of *Hordeum vulgare* L. and (B) in the presence of the alcohol ethoxylate triethylene glycol monododecyl ether (C_6E_3). Arrows on the left and right side of the spectra mark the limitations of the hyperfine splitting $2A_{max}$. The total scan range was 100 gauss.

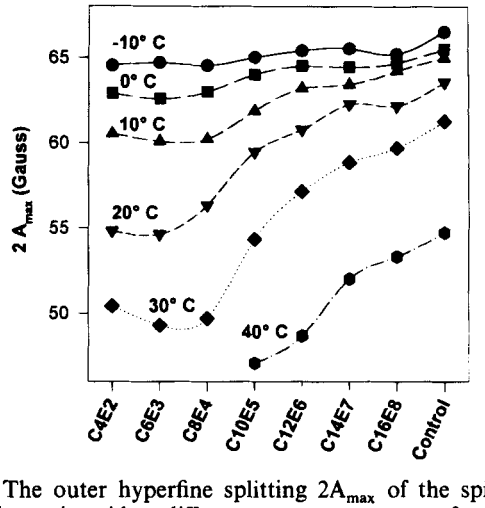

Fig. 4. The outer hyperfine splitting $2A_{max}$ of the spin label 5-doxyl stearic acid at different temperatures as a function of the investigated alcohol ethoxylates.

order of magnitude larger, ranging from 22-fold with $C_{16}E_8$ to 315-fold with C_8E_4. Plotting the effects of the respective alcohol ethoxylates on the mobilities of the two compounds PCP and $C_{24}AC$ as a function of the maximum equilibrium concentrations in the wax shows good linear correlations (Fig. 2).

At low temperatures, ESR spectra of both pure wax (Fig. 3A) and wax containing a defined amount of an alcohol ethoxylate (here C_6E_3; Fig. 3B) showed the typical appearance for a sample in the solid phase. The pronounced hyperfine splitting in the outer regions of the spectra indicated a strong restriction of the mobility of the spin probe. With increasing temperatures hyperfine splitting constantly moved from both sides towards the centre of the spectrum finally disappearing completely (Fig. 3). Plotting the outer hyperfine splitting at each temperature as a function of the respective alcohol ethoxylate, a characteristic distribution was obtained (Fig. 4). Samples containing alcohol ethoxylates of intermediate size showed most rapid decrease in the size of the hyperfine splitting, whereas samples with the smallest and the larger alcohol ethoxylates showed a significantly slower decrease in the $2A_{max}$ values (Fig. 4).

4 DISCUSSION

Experiments carried out in this study are based on the hypothesis that the observed increase of cuticular permeability in the presence of surfactants is due to a direct interaction of the surfactants with the waxy transport barrier of the cuticle. Results of several investigations recently, dealing with the effects of monodisperse alcohol ethoxylates on the permeability of isolated cuticular membranes,[21,22] on the sorption of monodisperse alcohol ethoxylates to cuticular membranes,[23] and on the interaction of a single alcohol ethoxylate with isolated cuticular wax,[12] strongly suggested this interpretation. In order to analyse this hypothesis, the following information had to be obtained: (i) what are the maximum equilibrium concentrations of monodisperse alcohol ethoxylates in cuticular wax in contact with an aqueous solution, (ii) to what extent are the mobilities of organic compounds in reconstituted cuticular wax increased by alcohol ethoxylates and is this a function of the concentration of surfactant in the wax and (iii) in which way do surfactants interact with cuticular wax leading to decreased barrier properties? Thus, the following discussion will deal with these three questions and try to find answers to them.

4.1 Sorption of alcohol ethoxylates in wax

As shown recently, investigating the sorption of alcohol ethoxylates by isolated cuticular membranes[23] and the sorption of a single alcohol ethoxylate by isolated cuticular wax,[12] the maximum amount of a surfactant that can be sorbed from aqueous solutions is limited by the CMC. This is due to the fact that it is solely the concentration of the monomers, remaining more or less constant above the CMC,[26] which forms the driving force for equilibrium sorption to cutin or wax. Since the CMC of the investigated surfactants significantly decreases with increasing size of the alcohol ethoxylates, the maximum concentration in the wax decreases from C_4E_2 to $C_{16}E_8$ (Table 2). Furthermore, as the concentration of monomers in the aqueous solution stays essentially constant above the CMC, K_{ww} can easily be estimated as the quotient of maximum concentration in the wax and the CMC (Table 2).

Results obtained here with isolated wax of barley leaves show that equilibrium sorption of the alcohol ethoxylates to cuticular wax (K_{ww}) is about the one order of magnitude lower than equilibrium sorption of the respective compounds to cutin (K_{cw}) (Table 2 and Fig. 1). This is in good accordance with results obtained recently comparing the sorption of various pesticides to isolated wax of barley[27] and of different conifer species[28] with sorption to isolated cuticles. In order to avoid confusion it should be mentioned that the K_{ww} of $C_{12}E_8$ measured here (104($\pm$27)) is about 50% lower than the value reported in a recent investigation (197($\pm$77)). However, the two values are statistically not different at the 95% level.[12]

Concerning the mechanisms of sorption either to wax or to cutin, no fundamental differences should be expected. Lipophilic organic chemicals like pesticides and amphiphilic surfactants like the alcohol ethoxylates do not seem to behave differently. The fact that K_{ww} was always about one order of magnitude lower than K_{cw} can be attributed to the physically different structures of wax and cutin. Wax, which is composed of amorphous and highly ordered, crystalline phases,[7,8] evidently offers significantly fewer sorption sites than the amorphous cutin polymer.

4.2 Effects of alcohol ethoxylates on mobilities of organic compounds in wax

The distribution of the effects obtained with PCP and $C_{24}AC$ and isolated wax are very similar to those which have been obtained recently with 2,4-D and isolated cuticular membranes.[21] Furthermore, in contrast to $C_{24}AC$, which is a linear long-chain aliphatic molecule, absolute values of the effects for the two comparable compounds PCP and 2,4-D, which are both chlorinated, aromatic pesticide molecules, are similar, even though they have been obtained using two different experimental systems. Thus, effects of the alcohol ethoxylates, as measured with isolated cuticles, can also be observed using isolated cuticular wax. From this com-

parison it may be concluded that it is justified to analyse the mechanisms of cuticular transport applying this artificial model of reconstituted cuticular wax, which forms the transport-limiting barrier of the intact plant cuticle. However, despite the good accordance between isolated wax and intact cuticles found in this study, it should be pointed out that wax/cutin interfaces may also be of some importance in cuticular transport, and cannot be studied applying this artificial experimental system.

Some alcohol ethoxylate homologues, especially those of intermediate size, are more effective than others. Plotting the maximum equilibrium concentration of alcohol ethoxylates in the wax as a function of their effects shows good correlations (Fig. 2), indicating unspecific wax/surfactant interactions. Their magnitude should mainly depend on the concentration of surfactant in the wax. This has recently been suggested when correlating the concentration of the same alcohol ethoxylates sorbed in cutin with their effects on 2,4-D mobility in isolated cuticles.[23] However, with $C_{24}AC$ (Fig. 2) and 2,4-D,[21] C_4E_2 did not fit into the correlations, since the amounts sorbed to the wax were far too high compared with the induced effects (Fig. 2). For 2,4-D this deviation can be explained by the fact that the aqueous concentration of C_4E_2 used in the desorption experiments was more than 20 times below the CMC of C_4E_2.[21]

4.3 ESR-studies of surfactant/wax interactions

The interaction of surfactants with the waxy transport barrier of cuticles has been studied using ESR-spectroscopy. Since spin labels are not typical aliphatic molecules, due to their bulky and polar nitroxyl-group, they will be located in the amporphous wax phase.[7,8] Thus they form an excellent label for the amorphous wax phase, where the diffusion of 'non-wax' molecules, such as pesticides, is supposed to take place.[10] Several investigations dealing with similar questions concerning transport properties of different biological membranes, such as human skin,[29] biomembranes of plants[30] as well as plant cuticles,[31] have recently utilised ESR-spectroscopy.

Comparing the spectrum of pure wax with the spectrum of wax containing C_6E_3 at a concentration corresponding to the maximum concentration in the wax at 25°C above the CMC, it is obvious that the surfactant significantly reduced the temperature at which the hyperfine splitting of the spectrum completely disappeared (Fig. 3). Whereas it is still detectable at 40°C with pure wax, it had vanished completely at 30°C when the wax sample contained the alcohol ethoxylate C_6E_3. This shows that a significant fraction of the spin probe is still in a rigid environment in the pure wax at 40°C (Fig. 3A), whereas in the presence of the C_6E_3, the spin probe is in a liquid environment, where it can freely rotate around its own axis (Fig. 3B).

When the hyperfine splitting ($2A_{max}$) is plotted at equal temperatures as a function of the individual monomers (Fig. 4), the distribution of the decrease of the hyperfine splitting closely resembles the distribution of the effects of the respective alcohol ethoxylates. Thus, the different effects which are induced by the different surfactants on the mobilities of the investigated compounds are based on different degrees of fluidity of the wax environment where diffusion takes place. With those surfactants that reach higher concentrations in the wax, the wax barrier is obviously in a more fluid state at given temperature compared with other surfactants.

4.4 Conclusion

The investigations presented above were intended to help understand basic mechanisms governing the interaction of surfactants with plant waxes and to contribute to an improved design for the formulation of plant-protecting agents. In contrast to this study, which investigated a 'non-evaporating' system, the most important difference between our experimental system and the field situation will be that spray droplets evaporate. Thus, under real conditions, foliar uptake occurs from highly concentrated residues, consisting of the formulation components and the active ingredient. Surfactant effects will become independent of CMC and will be related to ratios of amounts of surfactants and wax and to surfactant mobility in waxes.[32] This is a situation where other principles may be more important than the ones described here. Therefore, future work concerning the enhancement of foliar uptake by surfactants must include these more realistic aspects of evaporating spray droplets.

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